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MILTON WHITNEY, *Chief.*

THE RÔLE OF OXIDATION IN SOIL FERTILITY.

BY

OSWALD SCHREINER AND HOWARD S. REED.

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Soils,
Washington, D. C., September 22, 1908.

Sir: I have the honor to transmit herewith the manuscript of a technical paper entitled "The Rôle of Oxidation in Soil Fertility," by Oswald Schreiner and Howard S. Reed, of this Bureau. This article embodies the results of work carried out in the Bureau and contains important information on some little-understood factors involved in the study of soil fertility. The facts here presented emphasize the beneficial effects of tillage and other practices conducive to thorough aeration of the soil, although the present study has been mainly along the line of oxidation processes in the soil, induced by the roots of growing crops.

The oxidation by roots of crops growing in the soil is an agent in soil improvement which has until recently been almost overlooked. The mild but effective oxidation produced in this way is shown to have a very appreciable effect in aiding the decomposition of organic matter in the soil. The importance of the organic constituents of the soil, as shown by our recent investigations, makes these results especially interesting, and the material throws additional light upon the question of soil fertility. In accordance with your suggestion the manuscript has been gone over carefully with Assistant Secretary Hays, who authorizes me to state that he concurs in my recommendation for its publication. This will form No. 56 in the series of bulletins of the Bureau of Soils.

Respectfully,

Milton Whitney,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

CONTENTS.

THE RÔLE OF OXIDATION IN SOIL FERTILITY.

INTRODUCTION.

Oxidation is one of the most important processes in the soil, and while well recognized in the practical tillage of soils, the process itself and the forces which tend to promote oxidation have not been well understood nor much studied. Oxidation in soils may be produced by several different agencies, among which are the following:

Changes brought about by purely chemical processes in which the oxygen in the air attacks the soil compounds, producing higher oxidized forms. In this class should be included the interaction between chemical individuals of the same kind, resulting in the oxidation of one at the expense of the other—the process technically known as auto-oxidation. To this also should be added the influence of agents in the nature of fertilizers which have oxidizing properties, such as manganese or ferric compounds, nitrates, etc., as well as those which by changing the reaction of the soil increase the direct oxidation by atmospheric oxygen and oxidizing agents, which usually takes place more readily under alkaline conditions. The nascent production of nitric acid in the soil may also be a factor in the direct chemical oxidation of soil compounds.

Bacteria and the oxidizing enzymes which they produce are known to play an important part in oxygen fixation in soils, especially those relatively rich in organic matter, although the chemistry involved has not been extensively investigated and is but little understood.

The plant roots and the oxidizing enzymes produced by them in the soil constitute other agencies of oxidation which until recently were almost overlooked and even unknown. The mild but effective oxidation produced in this way by the roots of the growing crops and enzymes in the soil has a very appreciable effect in altering some of the soil constituents, and thus influencing soil fertility, through the action of added fertilizers and systems of rotation on this oxidizing power.

The present paper [a] embodies a series of studies upon the oxidizing powers of plant roots grown in aqueous extracts of soils and in

[a] The authors wish to acknowledge the valuable assistance rendered by Mr. J. J. Skinner, of this Bureau, in carrying out many of the experiments reported in this bulletin.

solutions of various compounds. The results, it is believed, throw some light on the action of plants upon the soil and indicate how soil conditions affect certain functions of the plant. Recent work in soil fertility has shown that in many cases the constituents of soil and of fertilizers, in addition to furnishing plant nutrients, perform a variety of other functions. These studies show the influence of constituents of soil and of fertilizers upon the important function of oxidation.

THE RÔLE OF OXIDATION IN PROMOTING CHEMICAL CHANGES IN SOILS.

The experiments which are presented in this paper show that plant roots are able to carry on active extra-cellular oxidation, chiefly by means of the enzymes which they secrete. From the standpoint of root excretions the study is of interest because it has often been supposed that the roots of growing plants excrete organic and inorganic acids which aid in the solution of soil minerals. The idea undoubtedly owes its prevalence largely to the experiments of Liebig[a] and of Sachs,[b] which demonstrated the corrosion of polished marble plates by growing plants. The more recent investigations upon the subject made by Czapek,[c] Kossowitch,[d] and Kunze[e] have shown, however, that very little acid is excreted by the roots of the higher plants, and that the results of the earlier workers were mainly due to the action of carbon dioxide.[f]

Although the solvent action of the roots upon soil constituents as a result of the acids secreted is slight, if any, this oxidizing power gives them an important action upon the soil. Whether they have the power to oxidize the inorganic constituents of the soil remains to be determined; but it has been shown that they are able to oxidize organic substances, such as the chromogens employed in these experiments, at a fairly rapid rate. If these substances are oxidized it is only logical to conclude that the organic substances occurring in the soils are also oxidized by the action of plant roots.

In the light of this oxidizing power, certain relations of plants to organic materials in the soil become of the highest interest. The toxicity of certain organic compounds has been shown by the writers[g] to be materially decreased by the addition of sodium nitrate or cal-

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[a] Ann. Chem. Pharm., **105**, 139 (1858).

[b] Bot. Ztg., **18**, 117 (1860).

[c] Jahrb. wiss. Bot., **29**, 321 (1896).

[d] Ann. Sci. Agron. [2], 8 (1), 220 (1903).

[e] Jahrb. wiss. Bot., **42**, 357 (1906).

[f] In this connection also see the recent work of Stoklasa and Ernest, Jahrb. wiss. Bot., **46**, 55 (1908).

[g] Jour. Am. Chem. Soc., **30**, 85 (1908); Bul. 40, Bureau of Soils, U. S. Dept. Agr., 1907.

cium carbonate to the cultures in which plants were grown. It was found that the toxicity of a vanillin solution was somewhat decreased when calcium carbonate was allowed to remain in contact with it for ten days; but a similar action was not observed when sodium nitrate was used in the same way. Also, when a preliminary set of plants was grown in solutions of certain compounds the activities of the plant roots were able to decrease the toxicity to a certain extent, provided the original concentration of the solution was not fatal to the plants. The preliminary set of plants undoubtedly absorbed and removed some of the toxic material from the solution, but the greatly diminished toxicity of the solutions as well as the formation of dyestuffs indicated that other changes had taken place. The greatest decrease in toxicity was obtained when sodium nitrate or calcium carbonate was added to the toxic solution at the time of installing the preliminary set of plants. In other words, the activity of the plant roots working in the presence of the inorganic salts was able to accomplish far more in the amelioration of toxic conditions than either agent taken singly could do. Upon further investigation it was found that the toxic compounds were entirely absent after the plants had grown in solutions to which the sodium nitrate and calcium carbonate had been added, while fairly large amounts were present in corresponding solutions in which plants had been grown a similar length of time, but without the addition of the inorganic salts.

In the light of the data presented in this paper showing the power of sodium nitrate and calcium carbonate to increase the oxidizing power of plants, the ameliorating influence of these salts as shown in the previous work is made more intelligible. It would seem from these data that the amelioration observed is largely to be referred to the increased oxidizing power of the plants caused by the addition of the inorganic salts, especially since it has been shown[a] that the highly oxidized form of certain toxic compounds are less likely to be injurious than the less oxidized forms.

The beneficial effects of processes which involve oxidation have been well shown in the publication cited by the effects of neurine, choline, and betaine. Neurine, which is very toxic to wheat plants, contains the ethylene group Choline, which is less injurious than neurine, contains the ethyl instead of the ethylene group and has one carbon atom oxidized to the primary alcohol stage. Betaine, which is nontoxic, shows still more oxidation by having the alcohol group oxidized to carboxyl, with subsequent splitting off of water.

Another example of the beneficial effect of oxidation may be cited from the publications already mentioned. It was shown that a

[a] Schreiner and Reed, Bot. Gaz., 45, 73 (1908); Bul. 47, Bureau of Soils, U. S. Dept. Agr., 1907.

tyrosine solution lost its injurious properties upon long standing with exposure to the air, the solution becoming dark colored by oxidation. The changes wrought seemed to be the same as those produced by the action of tyrosinase, which oxidizes tyrosine to homogentisic acid and then to several dark-colored compounds. When plants were grown in this oxidized solution it was found that the injurious properties of the tyrosine had not only disappeared, but that the solution was actually beneficial to plant growth.

These results are believed to have importance in explaining the value and action of the so-called green manure used in agricultural practice. When green manures are first applied to the soil, they may exert a slightly injurious effect, but after time enough has elapsed for the oxidation and decomposition of the vegetable matter the crop shows a beneficial effect resulting from the action of these products of oxidation and decomposition.

While it would be presumptuous to assume that decrease in toxic action always accompanies the introduction of oxygen into organic compounds, it is undoubtedly true that in a number of cases the more highly oxygenated compounds are less toxic than those containing less oxygen.

This oxidation of organic compounds is of additional interest in the light of recent investigations, which show that the cause of unproductivity in certain soils is due to the presence of harmful organic compounds.[a] The productivity of such soils may be improved by the removal of these harmful compounds. The beneficial effects of oxidation in such soils may be inferred from the results of thorough tillage, involving subdrainage and cultivation, since these operations promote aeration of the soil with subsequent increase in growth of roots and microorganisms. Under such conditions experience has shown that the organic substances in the soil are most completely converted into substances commonly known as humus. It is certain that the oxidizing activities of the soil and plant play a significant part in this important process.

From our present knowledge of the conditions that are necessary for the maintenance of fertility in agricultural soils it appears that the process of oxidation plays an important part in maintaining productivity. Part of the oxidation in the soil is undoubtedly accomplished by the activity of microorganisms, but the growing plant roots are also a factor. It must be left to future investigations to delineate the exact course of changes which the oxidation produces. Enough evidence is at hand, however, to demonstrate that maintenance of the most favorable soil conditions requires the oxidation of the organic soil

[a] Jour Am. Chem. Soc., **30**, 1295, 1599 (1908); Buls. 36, 40, 53, Bureau of Soils, U. S. Dept. Agr.

constituents to a comparatively high stage of oxidation. The beneficial effects of oxidation are well illustrated by the case of the organic bodies, neurine, choline, and betaine, and by that of tyrosine. The same relations hold for inorganic bodies, as illustrated by the fact that elements like nitrogen, phosphorus, and sulphur are suitable for nutrients only when present in the form of nitrates, phosphates, and sulphates; the nitrites, sulphites, and phosphites being toxic. In this connection it is extremely interesting to note the conditions under which animal toxins have been found to arise and in turn become nontoxic. Vaughan and Novy state:[a] "It would seem, as Brieger has already pointed out, that a certain quantity of oxygen is necessary to the formation of poisonous bases. Free supply of oxygen, on the other hand, invariably yields nontoxic ptomains."

PREVIOUS WORK.

The existence of an oxidation process in soils has long been known. Without apparently understanding the precise cause of this phenomenon, Liebig pointed out its importance for productive soils, and, according to the same author, the phenomenon had been earlier investigated by Ingenhouss and De Saussure.

Deherain and Demoussy,[b] in studying the process of oxidation of the organic matter in several soils, found that oxygen was always taken up and carbon dioxide set free. The oxidation was attributed by the authors to the activity of micro-organisms and to regular chemical action, with rather strong indications that the chemical action was due to enzymes.

Wollny[c] studied the rate of oxidation by estimating the amount of carbon dioxide produced by 100 grams of soil in a given time at a constant temperature. The oxidation was by him attributed mainly to the activity of micro-organisms. His results confirm those of Deherain and Demoussy and in addition showed that the presence of antiseptics, like mercuric bichloride, phenol, thymol, etc., decreased, if not inhibited, the process of oxidation. The processes of oxidation resulting in nitrification and the production of carbon dioxide were favored by slightly alkaline conditions, such as those secured by the addition of lime. The oxidation processes were retarded by the addition of chlorides and sulphates to the soil, but were favored by the addition of phosphates and nitrates.

Russell[d] has used an improved method for studying the problems attacked by the preceding investigators. He has shown that, gen-

[a] Cellular Toxins, p. 248, Philadelphia, 1902.

[b] Ann. agron., **22**, 305 (1896).

[c] Die Zersetzung der organischen Stoffe und die Humusbildungen, Heidelberg, 1897.

[d] Jour. Agr. Sci., **1**, 261 (1905).

erally speaking, the rate of oxidation is higher in fertile soils than in infertile soils, in the surface soil than in the subsoil. The rate of oxidation was found to increase with the temperature, the amount of water (up to a certain point), and the amount of calcium carbonate. The observed disappearance of oxygen and formation of carbon dioxide was attributed to the action of micro-organisms.

In a subsequent piece of work, Darbishire and Russell[a] have studied the effects of partial sterilization upon the oxidation by the soil. They found that heating the soil to 100° C., or treatment with volatile antiseptics, which were subsequently removed, led to a marked increase in the amount of oxygen absorbed. The increased oxidation was attributed to the removal of organisms which formerly competed with the oxidizing organisms without on their own part being beneficial to oxidation.

If the increased oxidation and increased plant growth are primarily the result of the removal of certain microorganisms it is still difficult to see why, in their experiments of partial sterilization with copper sulphate and mercuric chloride, decreased oxidation was observed in some soils and irregular increases in others. It is just as probable that the favorable influence on oxidation and plant growth are due to the destruction of toxic organic compounds which previously retarded both plant growth and oxidation, whether by microorganisms, enzymes, or other vital agencies. This assumption is certainly in harmony with the results obtained by Nobbe and Richter[b] by treating the soil with ether, carbon bisulphide, chloroform, and benzene, and they themselves reject the idea of amelioration through the action of these compounds on microorganisms.

In connection with observations that oxidation in the soil is due to the activities of microorganisms, it is important to note that recent bacteriological investigations[c] have demonstrated that certain soil bacteria under anaerobic conditions will oxidize hydrogen. Interesting in this connection is also the work of Potter[d] in showing a slow oxidation of amorphous carbon by bacteria. Chodat[e] has adduced evidence that the important action of nitrifying bacteria is largely, if not entirely, a process of oxidation by means of the nitroxidase which they produce.

On the other hand, there have been put forth some facts which indicate that part of the oxidation may be brought about by enzymes.

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[a] Jour. Agr. Sci., **2**, 305 (1907).

[b] Landw. Vers-Stat., **60**, 433 (1904).

[c] Kaserer, Centralbl. Bakt. (2), **16**, 681, 769 (1906); Nabokich and Lebedeff, ibid. (2), **17**, 350 (1906); Niklewski, ibid. (2), **20**, 469 (1908).

[d] Proc. Roy. Soc., B, **80**, 239 (1908).

[e] Bul. Herb. Boissier (2), **6**, 512 (1906).

Woods [a] found evidence that oxidase and peroxidase were present in the soil. Cameron and Bell [b] reported that many soils when shaken with an alcoholic solution of guaiac gave the blue color characteristic of oxidases.

Recent studies by König [c] furnish evidence of a catalytic power of soils due to the presence of an enzyme. He found that the different soils tested had the ability to liberate oxygen from hydrogen peroxide in addition to giving a blue coloration to a solution of paraphenylene diamine. This action was almost, if not entirely, destroyed by treating the soil with hydrocyanic acid, chloroform, and by dry or moist heat. The soils which were richest in humus had the greatest power to decompose hydrogen peroxide. König assumes that there is some catalytic action due to the colloidal substances present in the soil, since even the treatment with heat and hydrocyanic acid did not entirely inhibit the decomposition of hydrogen peroxide. In support of this assumption, König showed that the sesquioxides of manganese, iron, and chromium are able to effect a material production of oxygen from hydrogen peroxide, but other substances like apatite, feldspar, oxides of zinc, cadmium, and calcium, however, did not attack hydrogen peroxide.

The literature dealing with the oxidizing power of plant juices is already voluminous. Within recent years our knowledge of processes going on within the plant has been greatly extended by the studies which have been made upon oxidizing enzymes. Since several comprehensive and instructive summaries of work on this subject have appeared, among which may be mentioned those of Bach and of Czapek's Biochemie, it is unnecessary in the present paper to review in detail what has been done in this rapidly developing field.

The study of the oxidizing enzymes which are given off by the roots of plants—i. e., extra-cellular oxidation—has received less attention, and it is to this particular field that the present study belongs.

Molisch [d] appears to have been the first to demonstrate the oxidizing power of root secretions and to show their enzymatic nature. He found that the root secretion was capable of oxidizing various organic substances, such as guaiacol, pyrogallol, gallic acid. His work showed that there was considerable active secretion on the surface of growing roots and that this secretion had definite powers to effect changes in organic substances.

Czapek [e], in making a general study of root secretions, followed some of the investigations made previously by Molisch. From experi-

[a] Centralbl. für Bakt. (2) **5**, 745 (1899); Bul. 18, Bureau of Plant Industry, U. S. Dept. of Agr., 1902.

[b] Bul. 30, Bureau of Soils, U. S. Dept. of Agr., 1905.

[c] Landw. Vers.-Stat., **63**, 471 (1906); **66**, 401 (1907).

[d] Sitzungsber. Akad. wiss. Wien. Math. nat. Kl., **96**, 84 (1888).

[e] Jahrb. wiss. Bot., **29**, 321 (1896).

ments upon the action of seedling roots upon starch paste and sugar solutions, he regarded it probable that the growing roots produce only diastase or inverting ferments, although exact proof could not be offered. He believed, however, that the experiments of Molisch failed to prove the production of oxidizing enzymes by roots.

Subsequent work by Czapek [a] establishes the existence of an intracellular oxidase in the roots of Vicia seedlings. It was found that "a short time after the beginning of geotropic induction there appears a retardation of the normal destruction of tyrosine, to be recognized by an accumulation of homogentisic acid." The cause of this retardation is attributed to the development of a specific antioxidase which inhibits the normal activity of the oxidase of the root tip. In normally growing roots the action of the oxidase appears to play a very definite rôle in the intra-cellular activities.

The ideas of the oxidizing powers of roots set forth by Molisch are well corroborated by the investigations of Raciborski [b] upon the oxidizing powers of plant tissues.

In his work reagents were used which were so nearly nontoxic that they could be added to solutions in which plants were grown. In some experiments the reagents were added to water cultures containing the growing plants; in others, strips of filter paper which had been saturated with the reagent were applied to the surface of growing roots. The substances used for showing the oxidizing power of growing roots were alpha-naphthylamine, benzidine, phenolphthalin, ferrous ammonium sulphate, Barbadoes aloes, guaiac, phloridzin, pyrogallol, leucomethylene blue, etc.

The extracellular oxidation by the roots of the phanerogams studied was found to be strongly localized and limited to the absorbing surface of the root. The most intensive oxidation occurs in the region covered by the root hairs. After the death of the root hairs, as the root grows older, the oxidation becomes weaker (as shown by the less intense coloration) and vanishes in basipetal order. The short growing zone of the root between the root cap and the region of root hairs shows very little, if any, oxidation. The cells of the root cap behaved differently in different plants. In some there was a very weak oxidizing power, insignificant in comparison with that of the absorbing region of the root; in other plants the root cap showed no power to oxidize. This observation is the more interesting because Pfeffer [c] regarded the experiments of Molisch to lack proof that the

[a] Jahrb. wiss. Bot., **43**, 145, 361 (1906). For a concise summary and bibliography of the articles describing this work, see Czapek, Ann. of Bot., **19**, 75 (1905).

[b] Bul. Acad. Sci. Cracovie, 1905, pp. 338, 668, 693.

[c] Abhandl. kön. säch. Ges. Wiss., Leipzig, Math. phys. Kl., **15**, 375 (189).

guaiac-bluing power was due to living cells and not to the dead or dying cells of the root cap.

The oxidation which occurred in naphthylamine and benzidine solutions first appeared on the outer surfaces of the walls of the root hairs and epidermal cells, later in the wall itself, and finally in the outer layer of the ectoplasm. When roots were left for a long time in a solution of these chromogens, the entire protoplasm of the epidermal cells and root hairs gradually assumed the dark color of the oxidized chromogen, although it was not determined whether this color was due to the diffusion inward of the dye formed at the surface or to an actual intracellular oxidation.

MATERIAL AND METHODS.

The experiments described in succeeding pages consisted in studying the oxidizing power of wheat plants grown under various conditions in connection with soil-fertility investigations. It was necessary to grow the wheat plants used for experimentation in solutions, since in such cultures it is possible to observe the oxidation without disturbing the roots. For the study of soil conditions an aqueous extract was made by stirring 1 part soil with 5 parts distilled water for three minutes and filtering after thirty minutes through a Pasteur-Chamberland filter tube. It has been found that soil extracts prepared in this manner possess a plant-producing power similar to that of the soil from which they were made. In other words, fertile soils yield extracts which promote good plant growth, and infertile soils give extracts producing poor plant growth. The effect of fertilizers was studied by adding pure salts to the soil extract. In other cases where the action of certain salts was being studied, the plants were grown in dilute solutions of those salts.

The water used in making solutions and soil extract was the ordinary laboratory distilled water treated with carbon black. The water was distilled from a copper boiler, condensed in a block tin worm and collected in a tin-lined copper tank. This method of distillation gives very good water for ordinary chemical work, but does not free it from traces of volatile organic compounds which may exert a harmful action, as was described by Livingston [a] and the writers.[b] It has been found that these deleterious substances may be effectually removed by treating the distilled water with some finely divided solid which possesses a strong absorbing power, such as ferric hydrate, or carbon black. The procedure usually followed was to shake up a small quantity of the carbon black in the water and allow it to stand for thirty to sixty minutes. At the expiration of that time the water

[a] Bul. 36, Bureau of Soils, U. S. Dept. Agr., 1907.
[b] Bul. 40, Bureau of Soils, U. S. Dept. Agr., 1907.

was filtered through ordinary filter paper and was ready for use. This treatment has been found to be as efficient in producing physiologically pure water as redistillation with strong oxidizing agents like acid potassium bichromate or alkaline potassium permanganate.

The varieties of wheat used in the experiments were "Chul" and "Harvest Queen." The seeds were germinated on floating perforated plates, according to the method described by Livingston,[a] and in Bulletin 40 of this Bureau. The seedlings were transferred from the perforated germinating plates to the cultures just as the first true leaf was beginning to emerge from its sheath.

The plants were held in notches cut in the edge of a cork, as described by Livingston. In this way the seeds in which enzymes were acting upon reserve food materials were kept out of the solutions and the enzyme effects observed were ascribable to those from the roots.

Salt-mouth bottles, having a capacity of 250 c. c., were used as culture jars and 10 wheat plants were grown in each jar. In each test two cultures containing 20 wheat plants were usually employed, and comparison was made with an equal number of plants growing in pure distilled water under the same conditions. All experiments were conducted in a greenhouse. During the season of the year in which conditions were most favorable for growth, each experiment was continued for eight to twelve days before studying the oxidizing action of the plants, but during the cloudy winter weather the time was sometimes extended to fourteen or sixteen days.

In addition to determining the oxidizing power of the plants subjected to various treatments, their growth was estimated by recording the green weight and transpiration of each culture.[b]

SUBSTANCES CAPABLE OF SHOWING THE OXIDIZING POWER OF ROOTS.

Two classes of substances have been found useful in showing the oxidizing power of plant roots in solution cultures. The first class comprises certain soluble chromogens which yield, upon oxidation by the plant roots, insoluble colored compounds mainly deposited upon the surface of the roots. The oxidation is usually rapid enough to produce marked results before the surface extension of the roots disturbs the zonal distribution of the colors. The second class of chromogens consists of certain substances which give soluble coloring matters as the result of the oxidizing action of the roots. The oxidizing action may be shown by the change from a colorless to a colored compound or by a change from one color to another and distinctly different color.

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[a] Livingston, Plant World, **9**, 13 (1906).

[b] For a discussion of the value of these criteria the reader is referred to Livingston, Bot. Gaz., **40**, 178 (1905); Jensen, Bot. Gaz., **43**, 11 (1907); and Bul. 47, Bureau of Soils, U. S. Dept. Agr. 1907.

Compounds belonging to the first class which have been used in this work are alpha-naphthylamine, benzidine, vanillin, vanillic acid, and esculin.

Alpha-naphthylamine is only slightly soluble in water, but constitutes a good reagent for use in plant cultures, because its colorless solution is nontoxic, or nearly so, to plants. When oxidized by the roots of plants, or by reagents such as ferric chloride or silver nitrate, alpha-naphthylamine is converted into the insoluble, lavender-purple oxynaphthylamine. When the oxidation is performed by the growing roots of a plant, the oxynaphthylamine is deposited upon the surface of the roots in characteristic zones, as already described by Raciborski.[a] The root cap is slightly, if at all, colored; the zone of primary meristematic cells immediately back of the root cap is marked by a distinct narrow band of color; the zone of actively growing cells in the region of greatest elongation is not intensely colored; the more slowly growing portions of the root possess the purplish color, but it becomes less intense as one passes to the upper parts of the root.

The superior oxidizing power of the meristematic tissues of the plant is not only shown by the narrow zone of deep color formed on the primary meristem of the apical portion of the roots, but also by the small dots of color produced on that portion of the root from which secondary roots arise. If a wheat root 8 to 10 cm. in length is placed in a solution of naphthylamine it will exhibit, in addition to the deeper colored zones near the apex, dark purple spots at the places where secondary roots are forming and are about to break through the cortical layers of the primary root. If secondary roots are already present, they show the same zones of colors already described for the primary roots.

The concentrations of naphthylamine used in solution cultures are necessarily low on account of its slight solubility in water, but are sufficiently strong to show the oxidation. In ordinary practice 10 parts of naphthylamine to a million (10 mg. per liter of water) is a suitable concentration to use. This concentration will eventually retard the growth of wheat plants, but is not detrimental to growth in the length of time usually required to demonstrate the oxidizing powers of the plant roots. A concentration of 5 parts per million sometimes acts as a stimulant to growth.

Benzidine is another chromogen which is oxidized by plants and may advantageously be used to demonstrate their oxidizing action. It is only slightly soluble in water, but in weak, colorless solution it is readily oxidized by plant roots to an insoluble dye which gives the roots a blue-black or black appearance. Benzidine is slightly toxic to

[a] Bul. Acad. Sci. Cracovie, 1905, pp. 338, 668, 693.

plant growth, but does not cause pathological conditions within the time required for demonstrating the oxidizing power of the plant roots. A concentration of 5 parts benzidine to a million of water will give good results and does not injure wheat roots in twenty-four hours, although that concentration may eventually inhibit growth.

The effect of oxidation may easily be demonstrated by allowing the roots of wheat plants to grow in a 5 part per million solution of benzidine for twelve to twenty-four hours. The formation of colors in distinct zones is fully as striking as in the case of alpha-naphthylamine. As before, the root cap does not produce oxidation products, the primary meristem is marked by a narrow band of brown color, the zone of elongation is practically uncolored, whereas the portion of the root just above the zone of greatest elongation is entirely colored blue-black or black by the oxidation products.

Solutions of vanillin and vanillic acid act in much the same manner as those of naphthylamine or benzidine, but the concentrations required to demonstrate the oxidizing power of roots are quite strongly toxic.[a] Both substances are converted by the oxidizing action of the roots into a purple insoluble dye which stains the surface of the roots in the manner previously described. The concentration of vanillin in the solution most favorable for showing oxidation with wheat plants lies between 250 and 500 parts per million. A solution of this concentration will demonstrate the oxidizing power of the roots before the plants become seriously injured. To demonstrate the oxidizing power of roots with vanillic acid, a solution of the latter containing 25 to 50 parts per million should be used.

Esculin is another chromogen belonging to this class, but was found to be less suitable for this work. Esculin solutions when freshly prepared exhibit a blue fluorescence. After plant roots have grown for a few days in such a solution, the blue fluorescence is lost and the roots themselves are colored yellow as a result of their oxidizing activity, the dye formed being insoluble and remaining upon the surface of the roots where the greatest oxidation occurs. The concentrations necessary to demonstrate the oxidizing power of roots range from 500 to 1,000 parts per million and are eventually quite toxic to wheat plants.

The second class of chromogens, viz, those which are converted into soluble coloring matters, are in many respects more useful for oxidation studies than those belonging to the first class, because the intensity of the color, and hence the amount of oxidation, can be quantitatively expressed. The substances belonging to the second class which have been employed in this study are phenolphthalin,

[a] Bul. 47, Bureau of Soils, U. S. Dept. Agr., 1907; Proc. Am. Soc. Biol. Chem., **1**, 33 (1907); Bot. Gaz., **45**, 73 (1908).

aloin, and leuco-rosolic acid. Alcoholic solutions of guaiac were also used for various tests, but could not be put into solution cultures containing growing roots.

The value of phenolphthalin, a leuco compound prepared from phenolphthalein, as an indicator of oxidizing enzymes, has been demonstrated for plant work by Kastle[a] and by Raciborski.[b] Phenolphthalin is prepared by the method described by Baeyer,[c] which consists in reducing ordinary phenolphthalein with zinc dust and sodium hydroxide to phenolphthalin. The latter substance is oxidized back to phenolphthalein by the oxidizing power of the plant roots, a change which is readily demonstrated when the solution is rendered alkaline. The following procedure was observed in preparing this reagent: Weigh out 250 mg. of phenolphthalein, 3 grams sodium hydroxide, and 4 or 5 grams of zinc dust. Place all in a flask and add 100 to 150 c. c. of water. Place the flask on a sand bath and heat sufficiently to cause a rapid evolution of hydrogen without causing the contents of the flask to boil violently. The heating usually requires two to three hours to effect reduction of the phenolphthalein. The contents of the flask, after reduction is completed, may be filtered and rendered nearly neutral with hydrochloric acid and may then be used as indicator in the plant cultures. However, better results may be obtained by using phenolphthalin purified according to the method given by Baeyer. After purification the phenolphthalin is dissolved in N/10 or N/20 NaOH and a few cubic centimeters of the alkaline solution put into each culture. If quantitative results are desired, it is necessary to reduce all the solution cultures to neutrality or the same degree of alkalinity. A very slight degree of alkalinity is not usually harmful to plants within the duration of an experiment and is favorable to the process of oxidation. Phenolphthalin is slowly oxidized by mere contact with the air; therefore it is advisable to install controls which will allow the results to be corrected for this atmospheric oxidation. When the phenolphthalin is added to the solution cultures, a like quantity is therefore added to jars of distilled water equal in volume to the cultures. The amount of oxidation in these blanks is subtracted from what is observed in the plant cultures.

Plant cultures usually show striking results at the end of ten to twenty hours, depending somewhat upon the temperature and amount of root surface. At the end of the experiment the plants are removed from the cultures and all are rendered distinctly alkaline with

[a] Am. Chem. Jour., **26**, 526 (1901); Bul. 26, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Service, 1906.

[b] Bul. Acad. Sci. Cracovie, math.-nat. Cl., 1905, 338.

[c] Ann. Chem., **202**, 80 (1880).

sodium hydroxide solution and thus the red phenolphthalein color is developed.

The great advantage in the use of phenolphthalin to demonstrate the oxidizing power of roots lies in the fact that it is capable of yielding quantitative results. After the colors have been developed in the alkaline solution their intensities may be estimated by the aid of a colorimeter. In the work reported below the color intensities were estimated by means of the colorimeter previously described,[a] which permits of rapid and accurate readings. The colored solutions may be read against a standard phenolphthalein solution or against a standard Lovibond red glass slide.[b] The readings of the colorimetric tubes are inversely proportional to the color intensity and are easily reduced to their relative values.

Aloin is a substance which may be used to demonstrate the oxidizing power of roots in the same way as phenolphthalin is used. Aloin, or barbaloin, is the active principle of Barbadoes aloes and is obtained in the market in the form of a yellow powder, fairly soluble in water and serving well as an indicator of the oxidizing power of plants. At the concentrations used in our work it was not found to exert any toxic action upon plants. As a result of a limited investigation of the chemistry of aloin it seems that its value as an indicator of the oxidizing power of plants depends largely upon the content of iso-barbaloin.

When oxidized by the plant roots, the aloin solution is changed from a pale yellow color to a permanent deep wine-red color similar to that given by Klunge's reaction for iso-barbaloin. Klunge's reaction[c] consists in dissolving aloin (containing iso-barbaloin) in a 15 per cent sodium chloride solution and adding 5 c. c. of concentrated copper sulphate solution. Almost immediately the yellow, straw-colored solution begins to change to a permanent deep wine red. The change is hastened by warming the solution.

When experimenting with plant juices containing enzymes there appears to be a difference between the reactions to aqueous and alcoholic solutions of aloin. As the results of experiments described in detail in a subsequent section of this paper, it was found that an aqueous solution of aloin is a better indicator of the presence of oxidase, while an alcoholic solution of aloin is the better indicator of peroxidase.

Aloin, like phenolphthalin, should be added to neutral or faintly alkaline culture solutions, and where quantitative results are desired

[a] Jour. Am. Chem. Soc., **27**, 1192 (1905); Bul. 31, Bureau of Soils, U. S. Dept. Agr., 1906.

[b] Lovibond, Jour. Soc. Chem. Ind., **13**, 308 (1894); see also Schreiner, Pharm. Review, **19**, 61 (1901).

[c] Schweizerische Wochenschr. f. Pharm., **21**, 1 (1883); also Leger, Compt. rend., **131**, 55 (1900).

all solutions should be of the same degree of alkalinity. In all of our work aloin was added at the rate of 100 mg. of aloin to 250 c. c. of culture solution. If actively growing seedlings are used in a very faintly alkaline solution, a small amount of red color may be developed in an hour or two, but the experiments should be continued for twelve to twenty hours for the final observation. When certain inorganic salts were present in the culture solutions, the aloin red color was slightly modified. The addition of nitrates or previous treatment of the soil extracts with an absorbing agent gave the oxidized aloin a purplish tinge, resembling that of fresh fuchsin solution. The presence of calcium carbonate gave a purer red color, resembling alkanna or cochineal solution.

The fact that aloin is changed by oxidation from a light yellow to a deep red solution makes it somewhat more difficult to obtain colorimetric readings than in the case of phenolphthalin, where there is a change from a colorless to a red solution. It is nevertheless practical to use the colorimeter for measuring approximately the intensity of color in aloin solutions by arranging the solutions in the order of their apparent color intensities and using each solution first as an unknown and then as a standard for the next higher. For example, let No. 1, the weakest color, be the standard against which No. 2 is read. Then discard No. 1, set No. 2 at a convenient mark, and, using it as the standard, read No. 3. In turn No. 3 is used as the standard for No. 4, and so on. In this way one avoids the necessity of comparing a solution strongly tinged with yellow against a solution which contains little or no yellow tint. In any two solutions to be estimated the tints of yellow should not be greatly different.

Leuco-rosolic acid is another reagent which is useful for demonstrating the oxidizing power of plant juices[a] and plant roots. When a few cubic centimeters of a slightly alkaline, colorless solution are added to a culture containing plants, the leuco-rosolic acid is oxidized back to rosolic acid, the change being shown by the appearance of the red color. This reagent is not so generally useful as phenolphthalin and aloin, since it is more readily oxidized by mere contact with the air, as well as being more difficult to prepare.

PRELIMINARY EXPERIMENTS.

The first experiments were conducted for the purpose of ascertaining some general facts concerning the phenomenon of oxidation by the roots of seedlings, as well as to learn the methods best suited for studying oxidation in soil extracts. The experiments of Raciborski dealt with plants growing under what may be termed pure

[a] Kastle, J. H., Bul. 26, Hyg. Lab., U. S. Pub. Health and Mar. Hosp. Serv., p. 17 (1906).

culture conditions, and those of Kastle were concerned with the oxidizing power of plant extracts.

In the first experiment wheat seedlings 4 days old were placed in solutions of alpha-naphthylamine having concentrations of 1, 2, 5, and 10 parts per million, and in a solution of 5 parts per million benzidine. The experiment was set up at 4 p. m., and observations were made eighteen hours later. At the expiration of that time colors could be distinctly seen on the white surface of the wheat roots. The roots in the solution of 1 part per million naphthylamine were pale lavender; in 2 parts per million they were pronounced lavender, except at the root cap; in the 5 parts per million solution they were violet in the region occupied by the primary meristem, and in the region of the root hairs where growth of elongation occurs; the root-cap and a narrow zone just above the primary meristem were uncolored; in the 10 parts per million solution the roots showed the same colors as in that of 5 parts per million. The roots in the solution of 5 parts per million of benzidine showed their power of oxidation by the formation of brown-violet color distributed in the same manner as described for the roots which grew in the solutions of naphthylamine.

In order to learn whether the oxidizing powers of roots were affected by conditions which favor growth, and also whether the method used in the first experiment would show such differences, the following experiment was made: Three water cultures were made, in each of which an equal number of wheat seedlings of uniform age and size were employed. One culture was made with redistilled water, the second with an aqueous extract of a rich garden soil, the third with a dilute aqueous extract of well-decomposed stable manure. After the plants had grown for one day in these liquids the oxidizing powers of the plants were determined by transferring them to other bottles containing 2 parts per million of alpha-naphthylamine in distilled water. At the expiration of eighteen hours the intensity of the purple colors showed that the roots which had previously grown in the extract of garden soil had oxidized more naphthylamine than those which had grown in distilled water, and those which had grown in manure extract had oxidized more naphthylamine than those from the garden soil extract. At the end of twenty-four hours the differences in color intensity in the two cultures were still more marked.

The next experiment was an attempt to employ a method which would permit a more accurate quantitative expression of the oxidizing power of the roots. Two cultures of wheat seedlings were grown for five days in an extract of unproductive soil under the same conditions as two other cultures in an extract of rich garden soil. Each culture contained 60 c. c. of the respective soil extract. The oxidizing

power of the roots was, in this experiment, shown by using phenolphthalin. The phenolphthalin was prepared by the method given in a previous paragraph and 0.4 c. c. of the freshly prepared solution added to each culture of plants after they had grown five days in their respective solutions. Nineteen hours after adding the indicator all plants were removed from the cultures and the solutions rendered alkaline, thus producing the phenolphthalein color. The solutions were brought to the same volume by the addition of distilled water, and the relative amount of oxidation was measured by determining the color intensities of the different cultures.

The two cultures of poor soil gave readings of 40 and 42 divisions on the graduated tube against slide No. 2 (Lovibond system); the two cultures of rich garden soil gave readings of 14 and 24 divisions against slide No. 4 (Lovibond system). Averaging the readings and comparing the intensity of the colors, the oxidation in the poor lawn soil and in the rich garden soil stands in the ratio of 1 to 4, or more exactly as 19 to 82. This result indicated that a procedure based upon this method will give satisfactory quantitative results.

This method was further tested by another experiment, in which different beneficial treatments were applied to an extract of the unproductive soil used in the last experiment. The results of the last experiment showed that the oxidizing power of plants growing in solutions of different physiological properties varies considerably, but left the question open as to how much of the oxidation result might be due to plants and how much to the solution. In the present experiment, therefore, two of the four bottles in each set of solutions were left unplanted and their oxidizing powers measured along with those of the solutions which contained plants. The treatment applied consisted in adding fertilizer substances in the form of pure chemicals. Calcium carbonate was added at the rate of 2,000, and sodium nitrate at the rate of 50 parts per million, respectively. The cultures were put up August 24 and allowed to grow until August 28, when the amount of water transpired by each culture was ascertained and 3 c. c. of a freshly prepared phenolphthalin solution added to each bottle. After root oxidation had taken place the color of the phenolphthalein was brought out by adding a few drops of strong alkali to each culture and the intensities of the different solutions compared in the colorimeter. Table I presents the figures which give the relative amount of oxidation in the planted and unplanted solutions. When the phenolphthalin solution was added to the culture jars the same quantity was added to a jar of distilled water, which served as a control upon the oxidation incident to contact with atmospheric oxygen. The color intensity of this control was determined and subtracted from each of the other readings.

TABLE I.—*Relative oxidizing power of cultures and unplanted solutions of Takoma lawn soil extract, with and without the addition of fertilizer ingredients. Oxidizing power of plants grown in distilled water used as the basis of comparison.*

[P. p. m.=parts per million.]

No.	Culture.	Relative oxidation.
1	Distilled water (planted)	
2	Distilled water (planted) Average	100
3	Extract Takoma lawn soil (planted)	88
4	Same (planted)	74
5	Same (unplanted)	8
6	Same (unplanted)	19
7	Same +2,000 p. p. m. $CaCO_3$ (planted)	113
8	Same (planted)	63
9	Same (unplanted)	19
10	Same (unplanted)	25
11	Same +50 p. p. m. $NaNO_3$ (planted)	98
12	Same (planted)	63
13	Same (unplanted)	24
14	Same (unplanted)	17

The plants used in this experiment were quite young and the experiment was only continued for four days, a period rather too short for the maximum oxidation effect, as shown by subsequent experiments; nevertheless the results show that the different treatments affected the oxidizing powers. The plants grown in extracts of poor soil possessed less oxidizing power than the controls in distilled water, but the oxidizing power was increased by the addition of calcium carbonate. The addition of sodium nitrate did not show any marked increase in the oxidation in those solutions within the time of the experiment, although its effect as shown in later experiments is always to increase oxidation.

The point which is to be emphasized in this experiment and which has not been previously brought out is that the soil extract unplanted possesses a comparatively feeble power of oxidation, as shown by the use of phenolphthalin, and that the addition of calcium carbonate and sodium nitrate slightly increased this small oxidizing power.

An additional experiment was performed, using three different salts in distilled water. The results of this experiment, which are given in Table II, confirm those of the foregoing experiments in the soil extract. The cultures were made in duplicates, and the figures represent the averages of each pair.

TABLE II.—*Relative oxidizing power of cultures and unplanted solutions of three nutrient salts. Oxidizing power of plants grown in distilled water used as basis of comparison.*

[P. p. m.=parts per million.]

No.	Culture.	Relative oxidation.
1	Controls in distilled water (planted)	100
2	Solution, 50 p. p. m. NO_3 as $NaNO_3$ (planted)	282
3	Same (unplanted)	39
4	Solution, 35 p. p. m. K as KCl (planted)	72
5	Same (unplanted)	36
6	Solution, 50 p. p. m. PO_4 as $NaHPO_4$ (planted)	88
7	Same (unplanted)	21

The enzymotic nature of the oxidizing process was next investigated, using alcoholic guaiac. When alcoholic guaiac is added to a solution in which wheat roots have been growing for a time, evidence of the presence of peroxidase was obtained, but none for oxidase.

When young growing wheat roots are treated with a solution of alcoholic guaiac they instantly give a blue color, which deepens when hydrogen peroxide is added. This indicates that the cells of the plant root contain an oxidase, as Czapek has also shown.[a]

A word may be introduced at this place concerning the possible function of bacteria in producing oxidizing ferments which might accomplish some of the effects noted. It is, of course, possible that such organisms existed in the culture employed, since after filtering the extracts no especial precautions were taken to keep them sterile and microorganisms which were on the roots of the plants would be introduced into the solutions. That these microorganisms were responsible for any appreciable amount of oxidation in the experiments described in this paper is hardly possible. In the first place the solutions used were not well adapted for a very thrifty development of micro-organisms, as was shown by their freedom from turbidity, odors, or other indications. The definite zones of color produced when indicators like alpha-naphthylamine and benzidine were used and their close correspondence to definite zones of tissue in the root show that the oxidation is performed only by agents intimately connected with the roots. The colors due to oxidation were most intense on the regions of the root where growth was most active, whereas we would expect that the bacteria, if zonally distributed, would be more abundant on the dying cells of the root cap or the dismantled cortical layers of the older parts of the root. It seems, therefore, highly improbable that the oxidizing activities of microorganisms can be responsible to any appreciable extent for the results observed.

OXIDATION IN SOIL EXTRACTS.

Following the preliminary experiments already described, further experiments were made to study in more detail the oxidizing power

[a] Ann. of Bot., **19**, 75 (1905).

of plants grown in extracts of soil of different character. These experiments were chiefly designed to study the oxidizing powers of plants in extracts of good and poor soils, of extracts treated with absorbing agents, and in distillates of soil extracts.

The difference in the oxidizing power of plants in extracts of fertile and infertile soils is shown by the following experiments. In the first experiment an extract of Takoma lawn soil was compared with an extract of good Leonardtown loam. The former is a very unproductive soil and the latter is a much better and usually very productive soil. The oxidizing powers of the plants were determined by adding phenolphthalin to the cultures after the plants had grown in them for nine days. The growth and oxidizing powers of the plants are shown in Table III, relative to control cultures made in distilled water, which are represented as 100 in each case.

TABLE III.—*Comparative growth and oxidizing powers of plants in extracts of Takoma lawn soil and good Leonardtown loam. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Controls in distilled water	100	100
2	Extract Takoma lawn soil	33	72
3	Extract Leonardtown loam	50	286

In the comparatively short time of this experiment during cloudy winter weather, December 10 to 19, the plant growth as manifested by the figures for transpiration did not have time enough to show the relative productiveness of the two extracts, since it has usually been found that the Leonardtown loam extract produces in fourteen to eighteen days better plants than distilled water. The figures do show, however, a much greater oxidizing power in the plants grown in the extract of the more fertile soil even under these conditions.

Subsequent experiments were performed, the results of which corroborated the foregoing. In each case where growth was good there was also good oxidation; where growth indicated a poor soil extract the oxidation was small, as will be seen from Tables IV and V.

TABLE IV.—*Comparative growth and oxidizing powers of plants in extracts of poor sandy loam and garden loam. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Controls in distilled water	100	100
2	Extract poor sandy loam	77	103
3	Extract garden loam	125	275

TABLE V.—*Comparative growth and oxidizing powers of plants in extract of good and poor soils. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Controls in distilled water	100	100
2	Extract Arlington clay loam	75	107
3	Extract Clarksville silt loam	123	133
4	Extract Stockton peat	272	400

In all these experiments where direct comparisons are made between the extracts of soils that are so poor as to give less plant growth than pure distilled water and other extracts giving materially greater growth than pure distilled water, it appears to be unmistakably true that the cultures made of extracts of good, fertile soils possess much greater oxidizing powers than those made of extracts of soils of relatively less fertility.

The next question considered was concerned with the effect of treating the soil extract with absorbing agents. Treating the extracts of a more or less unproductive soil with carbon black or other good absorbing agent is usually beneficial to growth. This response seems to be quite general for all poor-soil extracts, although their response to other treatments may be quite different. Previous work in this laboratory [a] has shown that this ameliorating action is due to the removal of deleterious organic substances. Experiments were accordingly made in which a number of different soil extracts were treated with carbon black or ferric hydrate. The absorbing agent was shaken with the soil extract and filtered off at the expiration of a half hour in the same manner as the distilled water used in the experiments was prepared. The relative effect of this treatment upon growth and the oxidizing power of the plants is shown in Table VI, where the effect in the untreated-soil extract is in each case taken as 100.

[a] Buls. 28, 36, and 40, Bureau of Soils, U. S. Dept. Agr.; Breazeale, J. F., Bot. Gaz., **41**: 54 (1906).

TABLE VI.—*Effect of treatment with carbon black and ferric hydrate upon growth and oxidizing power of plants grown in extracts of various soils. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Arlington clay loam [a]	100	100
	Same, treated with carbon black	124	265
2	Takoma lawn soil [a]	100	100
	Same, treated with carbon black	137	100
3	Alloway clay [a]	100	100
	Same, treated with carbon black	116	117
4	Dunkirk sandy loam [a]	100	100
	Same, treated with carbon black	112	280
5	Miami silt loam [a]	100	100
	Same, treated with ferric hydrate	171	198
6	Marshall clay loam [b]	100	100
	Same, treated with carbon black	216	130
7	Clarksville silt loam [b]	100	100
	Same, treated with carbon black	450	227
8	Elkton silt loam [b]	100	100
	Same, treated with carbon black	179	317
9	Cecil fine sandy loam [b]	100	100
	Same, treated with carbon black	112	200
10	Hagerstown loam [b]	100	100
	Same, treated with carbon black	230	500
11	Cecil sandy loam [b]	100	100
	Same, treated with carbon black	193	241
12	Dutchess silt loam [b]	100	100
	Same, treated with carbon black	110	373
13	Poor sandy loam [b]	100	100
	Same, treated with ferric hydrate	170	534
14	Garden loam [b]	100	100
	Same, treated with ferric hydrate	136	313

[a] Phenolphthalin used in estimating oxidation.
[b] Aloin used in estimating oxidation.

It will be noted that in all but one of the soil extracts the effects of the treatment with an absorbing agent strongly increased the oxidizing powers of the plants subsequently grown in the extract. The treatment also increased the growth of the plants, as shown by the transpiration.

The increased oxidation, as well as the increased growth, points directly to the conclusion that the soil extracts have been so improved by the treatment given as to induce a more active functioning of processes necessary to secure the best conditions for growth. In the single case of No. 2 the growth was increased as a result of the treatment with carbon black, but the oxidation was not. This result was frequently obtained with the Takoma lawn soil; in some cases the oxidizing power was even slightly decreased as a result of treatment with absorbing agents, although growth was increased. No satisfactory explanation has as yet been obtained for this apparently exceptional action. It may be found upon further investigation that the lack of response was due to the presence of matter inhibiting oxidation, which was not removed by the carbon black. This question seems worthy of more study than we have been able to give it.

Extracts of poor soils sometimes contain volatile bodies of a deleterious nature which can be driven off by boiling and collected in the distillate. The writers have described the behavior of plants grown

[a] Bul. 40, Bureau of Soils, U. S. Dept. Agr., 1907.

in such distillates. In these cases the distillate usually exhibits the same toxic properties which the original extracts possessed and the residue is correspondingly improved.

To study the effects of these distillates upon the oxidizing powers of the plants, the following experiments were made: One liter of such a soil extract was placed in a distilling apparatus and distilled until 200 c.c. of distillate had passed over and been condensed. This fluid was made up to 500 c.c. by adding water and designated "First portion." When a second 200 c.c. of distillate had been collected, it was likewise made up to 500 c.c. and designated "Second portion." Cultures were made in each portion, together with controls in pure distilled water. At the end of a week the plants in the different solutions showed marked differences. The plants in the first portion of the distillate were very small and were dying; those in the second portion were much better; in fact were equal to the controls growing in distilled water. One hundred milligrams of aloin was added to each of the culture bottles and on the following day the amount of oxidation was noted by comparing the intensity of red color in each culture. The cultures in the "First portion" showed much less oxidation than either of the other two. The most oxidation appeared to have gone on in the cultures in the "Second portion," which was slightly in excess of that in the control cultures in pure distilled water.

The question was studied further and in a more quantitative manner by the following experiment: An extract of Elkton silt loam, having a volume of 750 c.c., was placed in a flask connected with a condenser and distilled. The distillate, amounting to 500 c.c., was collected in two portions of 250 c.c. each and used as a culture medium in which plants were grown, together with the residue in the distilling flask, which was diluted to its original volume and also used for growing plants. For comparison, cultures were also made in the original soil extract. The wheat plants were allowed to grow in the various solutions for 13 days and then their oxidizing powers were estimated by means of phenolphthalin. The growth and oxidation are shown in Table VII.

TABLE VII.—*Growth and oxidation in distillate and residue of Elkton silt loam. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Original soil extract untreated	100	100
2	First portion of distillate	53	20
3	Second portion of distillate	70	19
4	Residue after distillation, diluted to original volume	132	180

These results show that the distillates of this soil extract were less favorable for growth and oxidation than the original untreated soil extract, while the residue from distillation was materially improved. This seems to indicate that the original soil extract, like others which have been investigated,[a] contained a volatile toxic substance which inhibited oxidation by the roots, and that this substance was driven off by the process of distillation with resulting benefit to oxidation in the residue. Judging from the growth of the plants the first portion of the distillate contained a larger proportion of this deleterious substance than the second, although this smaller amount appears to be equally as deleterious to the oxidizing powers of the roots as the larger amount present in the first portion. The oxidizing power of the plants in the residue was much greater than in the distillates or in the original soil extract.

Evidently the oxidizing powers of the roots are affected by certain exterior conditions, since an improvement in the physiological properties of the soil extract results in increased oxidation and the presence of deleterious bodies results in decreased oxidation.

From the experimental results thus far presented it appears that the oxidizing power of the soil extracts themselves can be regarded as partly, but not mainly, responsible for the oxidation observed in the experiments. In one of the preliminary experiments reported in Table I it was shown that the soil extract after filtration through a Pasteur-Chamberland filter tube exhibited some oxidation, even when no plants were growing. It was likewise shown by the results in Table II that certain nutrient salts dissolved in distilled water were able to accomplish a material amount of oxidation without the presence of growing plants. It therefore seems unlikely that any considerable amount of oxidation was performed by microorganic growth. If we consider the result in this last experiment, where oxidation was increased in the residue from distillation after continued boiling, it seems that any extensive action, not only of microorganisms, but also of enzymes, must be precluded. In the soil, however, it is quite probable that both of these oxidizing factors would come into play, but it is quite certain that the oxidizing power of the roots would accomplish a considerable portion of the oxidation observed.

INFLUENCE OF VARIOUS SALTS UPON OXIDATION.

The next step in the investigation consisted in a study of the influence of various fertilizer ingredients upon the processes of oxidation already demonstrated. It is a fact recognized in the practice of agriculture that certain substances, such as lime or gypsum, employed

[a] Buls. 28, 36, and 40, Bureau of Soils, U. S. Dept. Agr.

as fertilizers produce beneficial results disproportionate to their possible function as nutrients. Also, that substances possessing high value as nutrients produce beneficial effects even when analysis shows that both soil and plant already contain comparatively large amounts of the identical element in question.

When this point was reached the inquiry naturally presented itself as to whether the various fertilizer ingredients used for increasing plant growth had an effect upon the oxidizing powers of the roots growing in solutions or soil extracts to which the salts had been added. For the purpose of studying these effects the chemically pure salts were added to extracts of soils which were known to be improved for plant growth by the addition of the salts in question. Wheat plants were grown in the soil extracts for eight to fourteen days by the culture method previously described; then the oxidizing power of the culture solutions (with the plants in them) was tested by adding a suitable chromogen and quantitatively determining the intensity of the colors resulting from the oxidation of the chromogen.

The first class of compounds whose action was studied was that containing nitrogen. The application of nitrogenous compounds to the medium usually produces a beneficial effect upon the plants grown in it. The agricultural problem of fertilization for many soils lies in the selection and application of suitable forms of nitrogen. It has been found profitable, accordingly, to study the effect of certain salts which are known to act more or less beneficially upon the growth of plants in soil and solution cultures. In practice the inorganic compounds most commonly used are sodium nitrate and ammonium sulphate.

A comparative study of the effects of different forms of nitrate was made upon Arlington clay loam, a soil which has been found to respond to the application of nitrates. The sodium, potassium, and calcium salts were used, selecting amounts which gave equal concentrations of NO_3 in each solution. The increase in oxidizing powers due to the addition of the nitrates is shown in Table VIII, where the increases in growth are also shown for the sake of comparison.

TABLE VIII.—*Relative growth and oxidizing power resulting from the addition of different forms of nitrate to extract of Arlington clay loam. Growth expressed in terms of relative transpiration.*

[P. p. m.=parts per million.]

No.	Culture.	Relative growth.	Relative oxidation.
1	Soil extract untreated	100	100
2	Same + 100 p. p. m. NO_3 as $NaNO_3$	116	180
3	Same + 100 p. p. m. NO_3 as KNO_3	132	104
4	Same + 100 p. p. m. NO_3 as $Ca(NO_3)_2$	198	112

The results of this experiment, which indicate that potassium nitrate is less favorable to the oxidation processes than sodium nitrate or calcium nitrate, was supplemented by another experiment with sodium and potassium nitrate upon three different soil extracts.

TABLE IX.—*Percentage increase in oxidation resulting from the addition of nitrate, as sodium nitrate and potassium nitrate, to various soil extracts.*

No.	Culture.	Increase due to addition of $NaNO_3$.	Increase due to addition of KNO_3.
1	Miami black clay loam	58	−20
2	Penn clay	50	66
3	Lyons silt loam	62	38
	Average	57	28

The outcome of these experiments is supported by the results of many which follow in subsequent pages, showing that potassium salts are less favorable to oxidation than either the sodium or calcium salts of the same acid. Concerning the comparative effects of sodium and calcium the data are not sufficient to allow of any general statements.

The comparative effects of various forms of nitrogen were shown by an experiment employing three different nitrates in the extract of Cecil sandy loam. The nitrate was added in each case at the rate of 50 parts per million of NO_3 (11.3 parts of N). By previous experiments it had been found that this soil was beneficially affected by the addition of nitrogenous fertilizers. The effect of these salts upon the growth and oxidizing power of plants grown in the soil extract is shown in Table X.

TABLE X.—*Effect of nitrates upon growth and oxidation in extract of Cecil sandy loam. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth	Relative oxidation.
1	Extract Cecil sandy loam	100	100
2	Same + sodium nitrate (11.3 p. p. m. N.)	197	147
3	Same + potassium nitrate (11.3 p. p. m. N)	152	90
4	Same + calcium nitrate (11.3 p. p. m. N)	170	280

In this experiment, as in the preceding, potassium nitrate, although materially increasing growth, was slightly unfavorable to oxidation; but sodium and calcium nitrate were both responsible for considerable increase in oxidation.

An experiment to show the comparative action of sodium nitrate and ammonium sulphate was made, using an extract of Arlington clay loam. This experiment ran eleven days, and the oxidation was estimated at its termination by the use of phenolphthalin. The per-

centage increases in growth and oxidation caused by the addition of sodium nitrate were 90 and 38, respectively; the increases due to the addition of ammonium sulphate were 16 and 13, respectively. From these data it appears that the beneficial action of ammonium sulphate in solution culture was slight in respect to both growth and oxidation.

In the next experiment upon the action of nitrogenous bodies, sodium nitrate was added to extracts of soils obtained from various localities. The list of soils is given in Table XI, where the effects of the nitrate upon growth and oxidation is expressed by figures, which show the percentage increase in each case over the untreated soil extract.

TABLE XI.—*Percentage increase in growth and oxidizing power of plants resulting from the addition of sodium nitrate to various soil extracts. Growth expressed in terms of relative transpiration.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Arlington clay loam (average of 4 experiments)	21	69
2	Miami black clay loam (average of 2 experiments)	19	36
3	Penn clay	−25	50
4	Lyons silt loam	81	62
5	Clarksville silt loam	40	12
6	Elkton silt loam	29	115
7	Cecil fine sandy loam	12	25
8	Hagerstown loam	12	49
9	Dutchess silt loam	4	− 34
10	Cecil sandy loam	109	221
	Average	30.2	60.5

With one exception the experiments summarized in the above table all show a marked increase in oxidizing activity accompanying the addition of sodium nitrate to the soil extract. This is especially striking in No. 3, where in the duration of the experiment the growth of the plant was not increased by the presence of the nitrate, but actually retarded; nevertheless the oxidation was in this case distinctly increased. The average result shows an increase in oxidation of 60.5 per cent as the effect of adding sodium nitrate to the soil extracts. It will be noted that the relation between the growth and oxidizing power is not a constant one, although when one is increased the other usually shows an increase. From our present knowledge of the processes affected by the presence of such a salt as sodium nitrate this result is not inconsistent. In the first place the nitrogen is capable of increasing growth because it is taken up by the plant and enters directly into the composition of important constituents of the protoplasm. By increasing growth and simultaneously accelerating various vital processes in the plant, it is quite probable that the production and secretion of peroxidase would be thereby increased, but it is probable that the salt would have also a

direct action upon the peroxidase in the solution, as will be subsequently shown.

The addition of calcium carbonate to culture solutions in which plants are growing usually results in a great increase in root development. This effect of calcium salts, which was first shown by Wolf,[a] has also been shown to hold for plants grown in soil extracts by the writers,[b] with the further observation that the transpiration of wheat plants is markedly increased by the addition of calcium carbonate.

In studying the effect of this salt upon the oxidizing power of roots, it was added to the extracts of various soils which responded in a greater or less degree to the application of lime in the field or in pots. The salt was added to the soil extracts at the rate of 2,000 parts of calcium carbonate to a million of solution at the time of installing the young plants. There was always some undissolved salt in the bottom of the culture bottles and this may have increased root growth slightly as a result of its power to absorb deleterious substances.

The percentage increase in growth and oxidation due to the addition of calcium carbonate to four different soil extracts are shown in Table XII.

TABLE XII.—*Percentage increase in growth and oxidation resulting from the addition of calcium carbonate to various soil extracts. Growth expressed in terms of relative transpiration.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Cecil fine sandy loam	34	150
2	Hagerstown loam	94	250
3	Dutchess silt loam	12	91
4	Cecil sandy loam	96	484

The addition of this substance materially increased not only the growth of the plants but also their oxidizing powers. The amount of increased oxidation seems more nearly comparable to that arising from treating the soil extracts with absorbing agents like carbon black and ferric hydrate than to any other treatment used in these experiments.

Further observations upon the oxidizing power of plants in solutions containing calcium carbonate showed that it caused more or less oxidation of the chromogen independently of plant action. A part of this alteration was due to the fact that in the slightly alkaline

[a] Landw. Vers-Stat., **6**, 203 (1864).

[b] Bul. 40, Bureau of Soils, U. S. Dept. Agr.; Jour. Am. Chem. Soc. **30**, 85 (1908).

solutions there was more rapid oxidation by atmospheric oxygen. The oxidation due to the mere presence of the calcium carbonate in the extract was, however, not found to be sufficient to account for the results which have been recorded in Table XII. As a means of comparison, experiments were made using other salts of calcium.

The nitrate, carbonate, and sulphate of calcium were added to different portions of an extract of Cecil sandy loam which were used as culture solutions for plants. The calcium nitrate was added at the rate of 66 parts per million, the calcium carbonate in excess of solubility as before, and the calcium sulphate at the rate of 200 parts per million. Table XIII presents the effect of these salts upon growth and oxidation, expressing that of the control as 100.

TABLE XIII.—*Relative growth and oxidation in extract of Cecil sandy loam due to the addition of calcium nitrate, carbonate, and sulphate. Growth expressed in terms of relative transpiration.*

[P. p. m.=parts per million.]

No.	Culture.	Relative growth.	Relative oxidation.
1	Soil extract untreated	100	100
2	Same + 66 p. p. m. $Ca(NO_3)_2$	173	207
3	Same + $CaCO_3$ (excess)	214	192
4	Same + 200 p. p. m. $CaSO_4$	194	497

From the above results it seems quite certain that the beneficial action of calcium carbonate upon oxidation is not primarily due to its alkalinity. It will be noted that calcium carbonate was responsible for somewhat greater growth than either of the other two calcium salts added, yet the relative oxidizing power of the cultures to which it was added was no greater than the others—in fact was slightly less than that of the calcium nitrate and much less than that of the neutral calcium sulphate.

In the study of the effect of potassium salts on oxidation these salts were added directly to the soil extracts at the rate of 35 parts of potassium per million of solution. Experiments were made with the two forms of potassium which are most frequently used in agricultural practice, viz, sulphate and chloride. The effect of these salts upon oxidation is given in Tables XIV and XV.

Potassium salts affect the growth of wheat plants differently from most other salts. It has been noted in all of the work in which wheat plants have been used that potassium sulphate or chloride usually favors the growth of the tops of the plant much more than the roots. In fact, the improvement in growth is often manifested only by the tops, while the roots make no better growth than in the untreated soil extracts. Coincident with this relation lies the diminished transpiration which is exhibited by the plants in solutions containing the potas-

sium salts, the amount of water transpired per unit of green weight produced being considerably lower than that required for a unit of green weight in the control cultures.[a] This effect of potassium salts is so great that in reporting this series of experiments it was found better to use the green weight of tops than the transpiration.

TABLE XIV.—*Percentage increase in growth and oxidation resulting from the addition of potassium as potassium sulphate to extracts of various soils. Growth expressed in terms of relative green weight of tops.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Arlington clay loam	2	−27
2	Miami black clay loam	25	7
3	Penn clay	5	17
4	Lyons silt loam (average of 3 experiments)	14	48
5	Merrimac fine sandy loam	1	−27
6	Marshall clay loam	27	0
7	Hagerstown loam	8	8
	Average	11.7	3.7

TABLE XV.—*Percentage increase in growth and oxidation resulting from the addition of potassium as potassium chloride to extracts of various soils. Growth expressed in terms of relative green weight of tops.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Arlington clay loam	26	27
2	Miami black clay loam	11	− 6
3	Penn clay	0	29
4	Lyons silt loam (average of three experiments)	31	58
5	Merrimac fine sandy loam	80	−21
6	Clarksville silt loam	8	− 9
7	Elkton silt loam	11	− 5
8	Cecil fine sandy loam	17	−45
9	Hagerstown loam (average of two experiments)	19	− 8
10	Cecil sandy loam	24	−20
	Average	22.7	0

The influence of potassium salts upon the oxidizing powers of plant cultures appears to differ in several ways from that of the two compounds just described. This may in part be the natural result of the way in which potassium salts affect the growth of wheat plants, as pointed out above.

The results reported in these tables show quite conclusively that the effect of potassium salts on the soil extracts named was not beneficial to oxidation. In a majority of the cases where potassium chloride was added there was less oxidation than in the untreated soil extract, although on the whole the response in plant growth was

[a] See also Hartwell, Wheeler, and Pember, 20th Report, R. I. Agr. Expt. Sta., p. 299 (1907).

greater than when potassium sulphate was applied to the soil extracts. The result of the ten experiments shows that potassium chloride was quite beneficial to growth, but its general effect was to diminish oxidation in these soil extracts. In the seven experiments upon the effect of potassium sulphate, practically the same conditions were found to prevail. It is true that oxidation was usually greater than in the untreated extracts, but in comparison with the increase produced by sodium nitrate or calcium carbonate the average effect must be regarded as very small.

The question arises, especially in view of the influence which acid conditions are later shown to exert upon these oxidizing enzymes, whether the small amount of oxidation may not be due to acid conditions in the soil extracts to which potassium salts were added. The plants, as the result of their power of selective absorption, withdrew potassium from the solution more rapidly than they drew sulphate or chlorides, thereby producing slightly acid conditions in the originally neutral solution. This question was answered in the negative by three different experiments. In the first place, by neutralizing the culture solution containing these salts before testing its oxidizing powers the oxidizing power was still low.

Another line of evidence was afforded by comparing the increases in oxidation due to the addition of three different salts of potassium, namely, the nitrate, chloride, and sulphate. When plants are grown in a solution containing potassium nitrate, they remove the acid radical more rapidly than the basic, although both are readily taken up. As a result the solution of KNO_3 becomes slightly alkaline, while KCl and K_2SO_4 solutions would, as just pointed out, become slightly acid.

Table XVI presents the results of a comparison between the results produced by the addition of these three salts to different soil extracts.

TABLE XVI.—*Comparison of the effects of potassium nitrate, sulphate, and chloride upon oxidation in various soil extracts.*

No.	Culture.	Percentage increase due to KNO_3.	Percentage increase due to KCl.	Percentage increase due to K_2SO_4.
1	Penn clay	33	29	17
2	Arlington clay loam	45	27	.....
3	Lyons silt loam (average of two experiments)	16	22	43
	Average	31	26	30

From the results here presented it appears that potassium chloride and sulphate, although producing slightly acid conditions, are not

materially less efficient in promoting the oxidizing power of plants in soil extracts than potassium nitrate when added to the same extracts. Moreover, these results are in harmony with those already given in connection with the comparison of potassium nitrate with other nitrates where it was also shown that the potassium had this same retarding effect upon oxidation.

As a final test an experiment was made in which potassium was furnished in the form of the hydroxide. At the dilution in which it was used N/1000 potassium hydroxide exerts no harmful action upon wheat plants. A solution of N/1000 sodium hydroxide was used in other cultures for comparison. The results of this experiment are shown in Table XVII.

TABLE XVII.—*Relative growth and oxidation resulting from the addition of potassium and sodium hydroxides to extract of Hagerstown loam. Growth expressed in terms of relative green weight.*

[P. p. m.=parts per million.]

No.	Culture.	Relative growth.	Relative oxidation.
1	Extract Hagerstown loam	100	100
2	Same+N/1000 KOH (39 p. p. m. K)	181	83
3	Same+N/1000 NaOH (23 p. p. m. Na)	165	112

The potassium hydroxide produced a large increase in growth in this experiment, but, as was the case with other potassium compounds, caused a small amount of oxidation. Sodium hydrate, though less beneficial to growth, is much more beneficial to oxidation.

It seems to be logical to conclude from these different experiments that the action of potassium is not highly conducive to oxidation in the majority of instances. This property appears to be quite independent of the salt which is used.

Many of the soils at our disposal were found to give material responses to the application of phosphates, both to the soil itself and to aqueous soil extracts. In most cases the increased growth of plants following the application of phosphates was shown by an improvement both in root and top growth, except in a few cases where the acid monocalcium phosphate was used. There the root growth was very poor, due to the acid condition of the solution.

The effect of three of the different phosphates was first determined by recording the growth and oxidizing power of plants grown in aqueous solutions of the respective phosphate salts. The solutions were made up so that each contained 50 parts per million of PO_4. Plants were installed in the cultures April 18 and grew until April 30. On the last-named day 100 mg. of aloin were added to each bottle and the red colors resulting from oxidation were estimated on

the following day. The growth and oxidation relative to the controls in distilled water are shown in Table XVIII, the control being taken as 100.

TABLE XVIII.—*Growth and oxidation resulting from the addition of various phosphates to distilled water. Growth expressed in terms of relative transpiration.*

[P. p. m.=parts per million.]

No.	Culture.	Relative growth.	Relative oxidation.
1	Control in distilled water	100	100
2	50 p. p. m. PO_4 as K_2HPO_4	114	62
3	50 p. p. m. PO_4 as Na_2HPO_4	150	140
4	50 p. p. m. PO_4 as $CaH_4(PO_4)_2$	77	59

The effect of the potassium phosphate, while distinctly beneficial to growth, was not beneficial to oxidation, but actually retarded it, wheras sodium phosphate in an equal amount was distinctly beneficial both to growth and oxidation. This result is in harmony with results given on a previous page, where the effect of sodium salts upon oxidation was shown to be more beneficial than that of the corresponding potassium salts. The acid calcium phosphate, which exerted a depressing effect on growth, also exerted a depressing effect upon oxidation, probably due to its acidity.

Following these observations, experiments were made for the purpose of determining the effect on oxidation produced by adding a phosphate to aqueous extracts of various soils. The salt chosen for this purpose was sodium phosphate, since it was found to act favorably upon growth. The sodium phosphate was added to the soil extracts at the rate of 50 parts of PO_4 per million. Table XIX shows the percentage gain in growth and oxidation in these experiments.

TABLE XIX.—*Percentage increase in growth and oxidation resulting from the addition of sodium phosphate to extracts of various soils. Growth expressed in terms of relative transpiration.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Miami black clay loam	22	131
2	Miami silt loam	54	— 33
3	Marshall clay loam	42	36
4	Clarksville silt loam	52	— 23
5	Sassafras silt loam	16	19
6	Elkton silt loam	77	92
7	Hagerstown loam	12	34
8	Dutchess silt loam	1	12
9	Cecil sandy loam	2	88
	Average	31	39.5

The addition of sodium phosphate appears, on the whole, to have been distinctly beneficial to oxidation as well as to growth. In two cases there was a material increase in oxidation, where there was only slight increase in growth, although it is entirely possible that this is due to the fact that these particular results were obtained in cloudy weather in January from experiments continued for only nine days. In a longer period the growth might also have been beneficially affected. In the case of Miami silt loam and Clarksville silt loam, the addition of sodium phosphate to the extracts produced a material increase in growth, but not in oxidation. The reason for this difference seems to be rather obscure, but is most capable of explanation upon the existence of a toxic compound in these soil extracts[a] whose deleterious action upon growth may have been largely overcome by the addition of the sodium phosphate, but still remained inhibitory to oxidation. In all the other cases the addition of sodium phosphate caused material increase in the oxidation accomplished by the plants in the soil extracts as well as in the plant growth. An average of all the results shows that sodium phosphate has a beneficial effect upon oxidation.

While chlorine is not one of the essential constituents of the ash of plants, it occurs in most plants and many agricultural soils are benefited by application of sodium chloride.

Wollny[b] found that the addition of chlorides to the soil diminished the rate of oxidation of organic material. The effect of the addition of potassium chloride to soil extracts was not materially different from the effect of the nitrate or sulphate as shown by Table XVI. The use of potassium chloride may be open to some objections, since potassium has been shown to depress the oxidizing power of roots; consequently sodium chloride was tried in several different soil extracts, as Table XX indicates.

TABLE XX.—*Percentage increase in growth and oxidation in various soil extracts, due to the addition of sodium chloride. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Miami black clay loam	22	94
2	Penn clay	19	80
3	Lyons silt loam (average of 3 experiments)	0	12
4	Dunkirk loam	− 1	−29
	Averages	10	39

[a] A toxic compound has been isolated from this sample of Clarksville silt loam and identified as dihydroxystearic acid. See Schreiner and Shorey, Bul. 53, Bureau of Soils, U. S. Dept. Agr., 1908; Jour. Am. Chem. Soc., **30**, 1599 (1908).

[b] Wollny, Die Zersetzung der organischen Stoffe und die Humusbildungen, Heidelberg, 1897.

The effect of sodium chloride appears, from these experiments, to be distinctly beneficial to the oxidizing power of plant roots and to confirm the results of the experiments in which potassium chloride was used. Although in one instance the sodium chloride decreased the oxidation, the average response for the four soil extracts was well marked and lay in the positive direction.

The action of sulphates upon oxidation has already been shown in Table XVI, where a comparison was made between the nitrate, chloride, and sulphate of potassium, as well as from Table XIV, where the results with potassium sulphate are shown. The effect of sulphate as sodium sulphate was studied in four different soil extracts. The result upon growth and oxidation is shown in Table XXI.

TABLE XXI.—*Percentage increase in growth and oxidation in various soil extracts due to the addition of sodium sulphate. Growth expressed in terms of relative transpiration.*

No.	Culture.	Percentage increase in growth.	Percentage increase in oxidation.
1	Clyde sandy loam	12	23
2	Penn clay	−27	86
3	Lyons silt loam (average of three experiments)	12	11
4	Dunkirk loam	− 1	−47
	Average	− 1	18

From the average result it would appear that sodium sulphate is but slightly beneficial to growth and oxidation, but the average is low on account of the retardation obtained in No. 4. If this result were omitted, the average would be 40, a figure indicating a distinctly beneficial effect on oxidation. In the case of the action of the sodium sulphate the retarding action of the potassium, as in Table XIV, is of course entirely obviated.

In general sodium sulphate has a slightly beneficial effect upon the oxidizing power of plants.

The data presented in the foregoing tables shows that the oxidizing powers of the plants were beneficially affected by the addition of certain salts to the cultures. In most cases where oxidation was increased, plant growth was also increased, but not always, nor indeed, in anything like a strict proportion.

Further consideration of the conditions of experimentation shows that the increased oxidation observed was not due in any considerable degree to the increased growth and oxidation by soil bacteria, since all the soil extracts were filtered through Pasteur-Chamberland tubes, which were frequently heated to a high temperature over a Bunsen flame. The number of soil bacteria in the solution would therefore be small and their action negligible for the purpose of the present discussion.

It seems more logical to conclude that the effect of the salts added was to activate the enzymes which caused the oxidation and thus to produce an acceleration. In view of the important activating effect which certain salts are known to exert upon digestive enzymes in the animal organism, it seems very probable that they play a similar rôle in this case. For example, the presence of calcium chloride accelerates the action of pancreatic juices. Recently it has been shown that salts may exercise a "protective" function for certain enzymes. Thus, Ford and Guthrie[a] found in course of a study of the amylase of resting barley that digestion of barley with sodium chloride, potassium sulphate, potassium dihydrogen phosphate, calcium chloride, calcium sulphate, glycine, asparagine, or alanine results in an increased diastatic activity. The authors hold the view that the enzymes would have little activity were it not for the small amounts of soluble salts in the extract. They found that when barley grains were soaked in water for about forty-eight hours, and then ground and extracted, the enzyme activity was low, owing, not to the solution of enzyme as the result of the first soaking, but to the removal of salts and other substances which help to dissolve and protect the amylase during the extraction of the ground grain.

Extracts containing papain, bromelin, or animal trypsin exhibited greater diastatic activity than extracts containing only the amylase, although such enzymes were proved to be devoid of amylolytic activity in themselves. It was found, however, that boiled solutions of papain gave a marked increase in activity without corresponding solution of nitrogenous material. The authors believe that the results indicate that the amphoteric proteins in the papain prevent the destruction of amylase which would occur under other conditions of extraction.

The length of the roots as a factor in influencing the amount of oxidation is a question which should apparently be considered, since in some cultures the roots attained greater development than in others. A consideration of the actual modus by which oxidation is accomplished, however, makes this question one of minor importance. It has already been shown that when chromogens which upon oxidation yield insoluble dyestuffs are employed, the greatest amount of oxidation is shown to occur on the apical region of the root and that the older portions of the roots accomplished relatively much less oxidation. Since none of the roots, in the time of the experiments, showed any extensive development of branches, it seems logical to conclude that the length of the roots in itself would have a very minor effect upon the amount of oxidation, but that their physiological condition would be of much greater importance.

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[a] Jour. Inst. Brewing, **14**, 61 (1908).

EFFECT OF TOXIC COMPOUNDS UPON OXIDATION.

Aside from the foregoing experiments, in which there were used extracts of soils which displayed toxic qualities toward plants, a few investigations were made upon the action of organic compounds whose toxic properties had been previously determined.

The organic compounds employed for this purpose were vanillin, cumarin, and santonin. The compounds were dissolved in distilled water and the resulting solutions used as cultures, taking care that the concentrations chosen were not so great as to be fatal to wheat plants within the duration of the experiment. Vanillin was used at the rate of 100 parts per million, cumarin 10 parts per million, and santonin in a saturated solution, which was nearly 100 parts per million. The growth of the plants as measured by transpiration and stated in figures, taking the growth of similar plants in distilled water as 100 in each case, was vanillin 63, cumarin 81, santonin 75. After the plants had grown in their respective solutions for from twelve to fourteen days, 100 mg. of aloin were added to each and the results noted on the following day. The results agreed in showing no color indications of oxidation in any of the cultures where the toxic compounds were present, although the roots growing in the control cultures in distilled water showed by the red color produced that a material amount of oxidation had been accomplished in them.

That the mere presence of organic materials did not inhibit the oxidation was shown by an experiment employing a solution of leucine which was slightly beneficial to the growth of wheat seedlings in solution cultures. Solutions of leucine containing 50 and 100 parts per million, producing an increase in growth over distilled water of 54 and 98 per cent, respectively, were very favorable to oxidation and produced a much deeper aloin red than the cultures in distilled water.

It can only be concluded, therefore, that the toxic organic compounds studied were deleterious to oxidation because of their toxic properties, and it appears that they were even more deleterious to oxidation than to plant growth.

The oxidizing action of the plants upon toxic organic substances is a phenomenon which has been pointed out by the authors in a previous paper[a] and will be referred to again later. The experiments presented in that paper also showed that the addition of sodium nitrate and calcium carbonate to solutions of toxic organic compounds went far toward decreasing their harmful effects, and in some cases overcame them entirely. That the organic salts and the physiological activities of the plants working together had accomplished the

[a] Jour. Am. Chem. Soc., **30**, 85 (1908).

destruction of toxic substances was shown by both plant growth and chemical tests. It now appears that while this destructive action of the toxic body by the plant is going on, the oxidizing power in the presence of an excess, as it were, of toxic body is greatly reduced and may even be entirely inhibited. The conclusion drawn from these experiments was that the plant roots are able to oxidize a certain amount of deleterious organic material and that the presence of salts which favor oxidation increases the ameliorating action of the plant.

This question was studied a little further by an experiment in which the oxidation in solutions of toxic material was observed. A solution of cumarin containing 10 parts per million, with and without the addition of fertilizer ingredients, was used as a medium for plant growth and subsequently examined for powers of oxidation. Sodium nitrate was added to one portion of the cultures at the rate of 50 parts of NO_3 per million, and calcium carbonate at the rate of 2,000 parts per million was added to another portion of the cultures. Wheat plants were installed in the cultures October 7 and grew until October 17. The oxidation was estimated by means of aloin. Table XXII gives the effect of these treatments upon growth and the oxidation in the toxic solutions and in control solutions to which no cumarin had been added. In each case growth and oxidation of the plants in distilled water are taken as 100.

TABLE XXII.—*Effect of sodium nitrate and calcium carbonate on growth and oxidation in solutions of cumarin. Growth expressed in terms of relative transpiration.*

[P. p. m. = parts per million.]

No.	Culture.	Relative growth.	Relative oxidation.
1	Distilled water	100	100
2	Same + $NaNO_3$	196	250
3	Same + $CaCO_3$	170	166
4	Cumarin 10 p. p. m	81	31
5	Same + $NaNO_3$	159	139
6	Same + $CaCO_3$	110	131

The results of this experiment show, in harmony with those of the previous work, that the addition of these fertilizer ingredients overcome to a large extent the deleterious effect of the cumarin upon growth, each one making the cumarin solution to which it was added a better medium for growth than distilled water. An inspection of the figures expressing the relative oxidation shows, however, that the addition of these salts produced relatively greater increases in oxidation than in growth. When sodium nitrate was added to cumarin the resulting growth was twice as great as where only cumarin was present; the oxidizing power, however, was increased over fourfold. In comparison with this effect it will be noted that the addition of

sodium nitrate to distilled water likewise increased the growth two-fold and increased the oxidizing powers two and a half times. It seems quite evident, therefore, that the ameliorating powers observed under the conditions of the experiment are to be referred to the increased oxidizing powers which are thereby brought about and the consequent diminution in amount and activity of the toxic material.

It may be noted in passing that Le Renard [a] found that nitrates had a greater antitoxic value than other acid radicals when Penicillium was grown in the presence of copper.

THE NATURE AND ACTIVITIES OF THE OXIDIZING ENZYMES.

Mention has been made of the enzymotic nature of the oxidizing action of the roots in preceding pages, and consideration will now be given to the nature of the enzyme or enzymes which bring about the oxidation. So far as known the oxidation effects observed were entirely due to the action of enzymes and not to the other activities connected with the growth of the roots themselves, for it was observed that under certain toxic conditions growth went on while oxidation was inhibited, and similarly, growth was sometimes enhanced by a treatment which did not change the oxidizing powers.

When a few drops of alcoholic guaiac are added to water, or a suitable solution of salts, in which wheat seedlings have grown for several days, there is sometimes a faint blue color indicating the presence of oxidase, but more often there is no blue color. When a drop of hydrogen peroxide is added, however, the liquid turns blue, giving a color varying from medium to very intense, depending somewhat upon the age of the seedlings, and the number of roots which have grown in the culture. The guaiac-peroxide reaction indicating a peroxidase is confirmed by phenolphthalin and aloin, both of which agree in showing the presence of peroxidase. When the roots of a young wheat plant are immersed in an alcoholic guaiac solution, they immediately turn blue, indicating that they are relatively rich in oxidase, although but little oxidase appears in the water in which they grew. This may be due to the retention of oxidase by the root cells during life, but when the outer cells are killed by the alcoholic guaiac the oxidase escapes and becomes evident through its reaction with guaiac. An aqueous extract of crushed roots shows strong oxidase reaction as well as peroxidase reaction. In the course of an examination of different parts of the young wheat plants, it was found that the partially depleted seeds showed a very strong oxidase reaction when guaiac was used, while the peroxidase reaction was relatively less than in the extract of crushed roots.

[a] Essai sur la valeur antitoxique de l'aliment complet et incomplet. Paris (1907).

When the solution in which wheat roots have been grown for some days is boiled for five to ten minutes and cooled the oxidase and peroxidase reactions disappear.

The temperature at which the peroxidase is destroyed as a result of applying heat was determined by heating a culture liquid which showed an active peroxidase action. The culture liquid was heated to successively high temperatures and held at each for five-minute periods. The temperature at which the enzymes appeared to be destroyed was 60° C., or very close thereto.

The culture liquid was examined for enzymes in a series of cultures of different ages to learn whether the enzyme reaction was equally strong in all. Wheat seeds were germinated on perforated cork plates floating on the surface of water in crystallizing dishes of 500 c. c. capacity. When cultures were on hand aged 2, 3, 4, 5, 6, and 7 days, respectively, tests were made with guaiac, alcoholic aloin, and phenolphthalin.

The tests with guaiac showed that the oxidase reaction which was weak in the 2 and 3 days' cultures was quite strong at 4 days. The tests with alcoholic aloin and phenolphthalin showed that the peroxidase reaction was strongest in the 6 and 4 day cultures, and considerably weaker in each of the others.

Certain phenomena observed in connection with the use of aloin in aqueous and alcoholic solutions suggested that they react differently with oxidases and peroxidases. Experiments were accordingly installed to test specifically the action of each solution. Two solutions of aloin were prepared: I contained 0.250 gram of aloin in 50 c. c. of water; II contained 0.250 gram of aloin in 50 c. c. of 95 per cent alcohol. One cubic centimeter of aloin solution, I or II, was added to 5 c. c. of liquid in test tubes according to the plan shown in Table XXIII. The tubes were prepared and aloin added at 2.45 p. m., on January 10, and the observations recorded in the third column of the table were made at 11 a. m., on January 11. The culture liquid when added to the tubes showed no oxidase but good peroxidase reaction with guaiac.

TABLE XXIII.—*Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing peroxidase.*

Nos.	Solution.	Color observed at end of 20¼ hours.
1 and 2	Unboiled liquid + 1 c. c. aqueous aloin	Pink.
3 and 4	Boiled liquid + 1 c. c. aqueous aloin	Do.
5 and 6	Distilled water + 1 c. c. aqueous aloin	Faint pink.
7 and 8	Unboiled liquid + 1 c. c. alcoholic aloin	Deep pink.
9 and 10	Boiled liquid + 1 c. c. alcoholic aloin	Yellow.
11 and 12	Distilled water + 1 c. c. alcoholic aloin	Do.

An inspection of these results shows that when only peroxidase is present aqueous aloin is not particularly applicable for demonstrating the presence of that enzyme, in the absence of growing plants, since there was the same development of pink color in boiled as in unboiled liquid. Alcoholic aloin, on the contrary, was changed to a deep pink in the unboiled liquid, but remained unchanged alike in the boiled liquid and in the distilled water.

The action of the different aloin solutions was next tested in liquids which also possessed a strong oxidase reaction. The roots of 10 wheat seedlings 12 days old were removed and crushed in a mortar with distilled water. The filtered liquid obtained from this source gave a strong reaction for oxidase when tested with guaiac. As before, 1 c. c. of aloin solution, I and II, was added to 5 c. c. of the root extract in test tubes, according to the plan shown in Table XXIV. The tubes were prepared and aloin added at 4.30 p. m., on January 13, and the observations recorded in the third column of the table were made at 11 a. m. on the following day.

TABLE XXIV.—*Comparative reaction of aqueous and alcoholic solutions of aloin to a liquid containing oxidase.*

No.	Solution.	Color observed 11 a. m. Jan. 11.
1, 2, 3	Root extract+1 c. c. aqueous aloin	Red.
4, 5	Distilled water+1 c. c. aqueous aloin	Faint pink.
6, 7, 8	Root extaact+1 c. c. alcoholic aloin	Pronounced pink.
9, 10	Distilled water+1 c. c. alcoholic aloin	Faint pink.

The results of these experiments supplement those of the foregoing, in which a peroxidase liquid was used, by demonstrating that the oxidase caused a much greater conversion of aloin to "aloin red" with the aqueous than with the alcoholic solutions of aloin. There was in the root extract a distinct peroxidase reaction to guaiac in addition to the oxidase reaction, and it is only natural that in tubes 6, 7, and 8 there should be some development of color when alcoholic aloin was added.

It is evident, from the above results, that in the absence of living plant roots aqueous aloin is principally a reagent for oxidase and alcoholic aloin for peroxidase. In the experiments where plants are employed it is however needless to say that only aqueous solutions of aloin can be used. But there again the living roots may introduce factors which cause peroxidase reaction with aqueous aloin.

Aloin and phenolphthalin, having shown their usefulness as indicators of enzyme action, several other substances were investigated for comparison. Leuco-rosolic acid was prepared by reducing rosolic acid with zinc dust in alkaline solution. When reduction was practically complete the solution was filtered and neutralized with

hydrochloric acid, then rendered slightly alkaline with sodium hydroxide. One cubic centimeter of this solution was added to three different liquids; (I) liquid from culture 6 days old; (II) the same liquid after having been boiled ten minutes; (III) distilled water. When examined twenty-four hours later I was pronounced rose-red, while II and III were merely faint pink, which indicates that leucorosolic acid is capable of showing the action of these oxidizing enzymes.

Attempts were made to use ferrous ammonium sulphate and potassium iodide as indicators of the oxidizing powers of plants by putting small amounts into cultures containing living plants. Ferrous ammonium sulphate was, in the space of time of the experiment, oxidized by mere contact with the atmospheric oxygen, and was therefore discarded as an indicator. Potassium iodide was not oxidized to free iodine, as Raciborski[a] has also found.

EFFECT OF DIFFERENT CONDITIONS IN THE SOLUTIONS UPON THE ACTIVITY OF THE ENZYMES.

Mention has previously been made of instances where the variation in oxidation appeared to be partly due to the acidity or alkalinity of the solution used as a culture liquid. In such cases the growth of the plant roots was affected when the conditions of alkalinity or acidity were very great. The effect is the more harmful when young seedlings are put into such solutions, because at the beginning of the experiment, when the plants are very tender, the acidity or alkalinity is greatest and gradually diminishes during the progress of the experiment. In investigating the effect of acid or alkaline conditions in the culture media, instead of using either alkaline or acid solutions at the start, a method was used whereby the originally neutral solutions became acid or alkaline as a result of the selective absorption of the plant in withdrawing nutrients from the solution.[b] It has been demonstrated by Kohn and Czapek[c] that fungi may render their culture media alkaline or acid as a result of their selective absorption, whereby an acid or a basic radical is removed more rapidly than the radical to which it is linked. Reed[d] has observed a similar action for the higher plants and pointed out its bearing upon the composition of nutrient solutions.

Solutions were made up from salts whose radicals are differently absorbed by growing plants, e. g., calcium nitrate and potassium sulphate. Where calcium nitrate is furnished, the plant takes up NO_3

[a] Bul. Acad. Sci., Cracovie, 668. 1905.

[b] See Cameron, Rept. 71, U. S. Dept. Agr., 1902, p. 67; Buls. 30 and 41, Bureau of Soils, U. S. Dept. Agr., 1905.

[c] Beitr. Chem. Phys. u. Path., 8, 302 (1906).

[d] Ann. of Bot., 21, 501 (1907).

more rapidly than Ca, with the result that the solution becomes increasingly alkaline. In the case of potassium sulphate, the plants take up K more rapidly than SO_4, with the result that the solution becomes acid. In the experiments which were made upon this problem an attempt was made to determine the acidity or alkalinity of the solutions when the experiment was terminated. A measured quantity of solution was boiled in a platinum vessel to drive off CO_2 and then titrated. The results of these determinations are shown with the other results in Table XXV.

TABLE XXV.—*Oxidation and growth of wheat plants in solutions which became acid or alkaline as the result of plant growth. Relative growth measured by transpiration.*

[P. p. m.=parts per million.]

No.	Culture.	Relative growth.	Acidity.	Alkalinity.	Relative oxidation.
1	Control in distilled water	100			100
2	30 p. p. m. Ca as $Ca(NO_3)_2$	174		N/5000	401
3	30 p. p. m. Ca as $CaCl_2$	112		N/7500	107
4	30 p. p. m. Ca as $CaCO_3$	123		N/7500	175
5	66 p. p. m. SO_4 as $(NH_4)_2SO_4$	46	N/7500		102
6	66 p. p. m. SO_4 as K_2SO_4	78	N/10000		100
7	100 p. p. m. NO_3 as $NaNO_3$	201		N/5000	401
8	35 p. p. m. K as KCl	92	Neutral.	Neutral.	97
9	100 p. p. m. NO_3 as $NaNO_3$ (N/620) 63 p. p. m. K as KCl (N/620)	283		N/10000	638
10	100 p. p. m. NO_3 63 p. p. m. K as KNO_3 (N/620) 63 p. p. m. K as K_2HPO_4 (N/620)	295		N/20000	250

These results show that six of the nine solutions became alkaline, two became acid, and one remained neutral. Growth and oxidation were less in the acid solutions than in those which became alkaline, although in the case of calcium chloride the result was quite low. In the case of potassium sulphate and potassium chloride a part of the depression may be due to the effect of the potassium, which usually fails to increase, materially, oxidation, but such is not the case with ammonium sulphate. Neither is it probable that the sulphate radical is the depressing factor, since calcium sulphate compares favorably with calcium nitrate in its effect upon oxidation, as was shown in Table XIII. The more favorable effect of No. 2 in Table XXV upon oxidation over Nos. 3 and 4 is probably to be attributed to the presence of nitrate, which likewise appears to be responsible for a material increase in growth. The greater oxidation accomplished by No. 9 over No. 10 is probably not to be attributed to the presence of Cl, but to the smaller amount of K present in No. 9.

On the whole, it appears that the oxidation is affected to a certain extent by conditions of acidity or alkalinity arising in the culture medium, but oxidation seems to be more materially affected by the specific action of the salts and their elements in the solution.

The effect of acid and alkaline conditions upon the activity of peroxidase was investigated by the following experiment in which

alcoholic aloin was used as the indicator. A liquid showing strong peroxidase action was taken from a pan in which several hundred seven-day-old wheat seedlings were growing. Various amounts of N/50 HCl and N/50 NaOH were added to a set of tubes, each containing 10 c. c. of the culture liquid and 1 c. c. of alcoholic aloin solution, added at 3 p. m., January 13. Table XXVI shows the amount of acid or alkali added in each tube, and gives the record of the colors observed at 11 a. m. the following day.

TABLE XXVI.—*Effect of acid and alkaline conditions upon the activity of peroxidase in the absence of plants.*

No.	Culture.	Color observed at end of 20 hours.
1	10 c. c. culture liquid +0.1 c. c. N/50 HCl	Faint pink.
2	Same +0.2 c. c. N/50 HCl	Do.
3	Same +0.5 c. c. N/50 HCl	No change.
4	Same +0.7 c. c. N/50 HCl	Do.
5	Same +1.0 c. c. N/50 HCl	Do.
6	Same +0.1 c. c. N/50 NaOH	Red.
7	Same +0.2 c. c. N/50 NaOH	Wine red.
8	Same +0.5 c. c. N/50 NaOH	Deep wine red.
9	Same +0.7 c. c. N/50 NaOH	Do.
10	Same +1.0 c. c. N/50 NaOH	Do.
11	Same neutral to litmus solution	Deep pink.
12	Same neutral to litmus solution	Do.

From these results it can only be concluded that a slightly alkaline medium is most favorable for this peroxidase reaction. It will be remembered that Wollny[a] found also that the oxidation processes in the soil were distinctly favored by slightly alkaline conditions.

The effect of putrefactive processes upon oxidation is another question which was briefly investigated. It has been observed that when a number of seedlings were placed without any support into water (the entire root system, seed and lower part of the plant being thus submerged) which contained aloin, the red color first produced subsequently disappeared. An experiment was accordingly planned to learn whether oxidation phenomena would be affected when the seeds were submerged and gave rise to products of putrefaction. Twelve cultures of wheat plants were prepared and allowed to grow four days in tap water. In one-third of the cultures the seedlings were adjusted in the notched corks so that only the root systems of the plants were submerged; in one-third of the cultures the seedlings were lowered so that the seeds were also submerged, and one-third had the seedlings entirely submerged. On the fourth day 100 mg. of aloin were added to each culture jar, and they were examined twenty-four hours later with reference to the production of colors. It was found that the cultures planted with only the root systems submerged showed a very considerable amount of oxidation, but in those where

[a] Die Zersetzung der organischen Stoffe und die Humusbildungen, Heidelberg, 1897.

the seeds or entire plants were submerged there was none of the red color produced by oxidation. In these cultures where no oxidation was shown there were putrefactive processes at work, a fact which is taken to mean that the oxidation effects are not observed when putrefaction processes occur. Whether this inhibition of oxidation is due to putrefactive processes or to a perverted metabolism of the plant which is functioning under somewhat anaerobic conditions remains undecided.

That the oxidizing power of the plant was not destroyed is shown by the fact that by raising the seeds out of the culture water and refilling the jars with fresh tap water containing aloin, the characteristic oxidation occurred.

The foregoing experiments raised a question as to the amount of oxidation which occurs in poorly drained soils where anaerobic and putrefactive processes are known to exist. In investigating this question, two crops of wheat seedlings were grown in Arlington clay loam in paraffined wire pots, giving the pots different amounts of water.

Lot I of the pots was taken at the optimum water content of the soil. The soil in lot II was kept saturated with water from the start and the soil in lot III was saturated after the wheat seedlings were up. The relative green weight of the first crop of wheat plants, which grew from February 18 to March 14, was: I, 100; II, 111; III, 104. The relative weight of the second crop, grown from March 17 to April 8, was: I, 100; II, 67; III, 116. Extracts of these soils were made and wheat plants were grown eleven days in the various extracts. At the end of that time the growth and oxidizing power of the plants in the different solutions were determined, with the result shown in Table XXVII.

TABLE XXVII.—*Growth and oxidation in extracts of soil of varying moisture content. Growth expressed in terms of relative transpiration.*

No.	Culture.	Relative growth.	Relative oxidation.
1	Soil kept at optimum	100	100
2	Soil kept at saturation	78	69
3	Soils saturated after plants were up	151	11

These results show that the effects of the poor drainage conditions appear to be much more marked upon oxidation than upon growth. The soil which was kept at optimum and only saturated after the plants had started seemed to remain favorable to growth in the pots and in the extracts, but its extract was plainly not favorable to oxidation. In regard to the increase of growth, it should be remembered that this lot of soil was alternately very wet and dry during the course of the experiment.

SUMMARY.

(1) Roots of growing plants exhibit an extracellular oxidizing power which may be demonstrated by the use of suitable chromogens in nutrient solutions or soil extracts.

(2) The oxidizing power appears to be most energetic in the region of the root where root hairs are found, and to decrease gradually in activity as that portion of the root becomes older.

(3) The oxidizing power of plants grown in extracts of productive soils is greater than that of plants grown in extracts of unproductive soils.

(4) Treating the soil extracts with an absorbing agent is usually beneficial to oxidation.

(5) The distillate of a poor soil extract which contains volatile toxic compounds was less favorable to oxidation than the residue remaining from distillation.

(6) The process of oxidation is usually accelerated by the addition of nitrates to an aqueous soil extract. The addition of ammonium sulphate is less beneficial to oxidation than the addition of an equal amount of nitrogen in the form of nitrate.

(7) Calcium salts were found to increase the amount of oxidation in cultures to which they were added.

(8) The addition of potassium salts was not generally beneficial to the processes of oxidation. In some cases their presence caused a material retardation of the oxidation. The most of the retardation was due to the action of the potassium itself and not to the formation of acid conditions in the solution. Sodium or ammonium salts of the same acid were more favorable to oxidation than the corresponding potassium salt.

(9) Phosphates usually produced material increases in the oxidation in solutions to which they were added.

(10) Chlorides and sulphates, when combined with a suitable base, like sodium, are somewhat beneficial to oxidation, but are not as favorable as the corresponding nitrate would be.

(11) The presence of toxic organic substances in solution was extremely deleterious to the oxidizing power of the plants. The oxidizing power of the plants, especially in the presence of nitrates, was able to alleviate the toxicity of such solutions.

(12) The process of oxidation by roots is largely, if not entirely, due to the activity of a peroxidase produced by the roots. This oxidizing enzyme is most active in neutral or slightly alkaline solutions. The activity of the enzyme may be inhibited by the presence of acid and also by the conditions in solutions where putrefaction processes occur.

(13) This oxidation by roots has considerable agricultural interest, since processes promoting oxidation play a large part in the best methods of soil cultivation and tillage.

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